Title
Assignment of the 2P domain, acid-sensitive potassium channel OAT1 gene KCNK3 to human chromosome bands 2p24.1-->p23.3 and murine 5B by in situ hybridization.

Journal
Cytogenetics and cell genetics, 86(3-4)

ISSN
0301-0171

Authors
Manjunath, NA
Bray-Ward, P
Goldstein, SA
et al.

Publication Date
1999

DOI
10.1159/000015349

Cytogenet Cell Genet 86:242–243 (1999)

Cytogenetics and Cell Genetics

Assignment[1] of the 2P domain, acid-sensitive potassium channel OAT1 gene KCNK3 to human chromosome bands 2p24.1→p23.3 and murine 5B by in situ hybridization

N.A. Manjunath,[a] P. Bray-Ward,[a] S.A.N. Goldstein,[b] and P.G. Gallagher[b]

Departments of [a] Genetics and [b] Pediatrics, Yale University School of Medicine, New Haven, CT (USA)

[1] This is a more precise localization of the human gene previously mapped to 2p by Lesage and Lazdunski (1998) and to our knowledge this is the first time the murine gene has been mapped.

Rationale and significance

Potassium channels are a diverse group of proteins with wide cellular and tissue distribution that serve a broad spectrum of physiologic functions and participate in a variety of cellular processes (Jan and Jan, 1997). They exhibit marked genetic, biochemical, structural, and functional diversity and defects in these channels have been associated with various genetic disorders. Potassium channels share a common pore-forming P domain that determines channel conductance, selectivity, and sensitivity to open-channel blockade. Recently, a novel family of potassium channels differing in cellular, biochemical, structural, and functional characteristics has been identified that contains two P domains in tandem (Goldstein et al., 1998). Members of this family, OAT channels, so named for their Open, Acid-sensitive Two P domain characteristics, have been identified in mouse, rat, and man (Kim et al., 1998; Lopes et al., 1998; Duprat et al 1997; Leonoudakis et al., 1998). Localization and functional characterization of OAT1 suggest that it may participate in cardiac action potential amplitude and duration, and may play a role in either inherited or acquired disorders of cardiac conduction (Lopes et al., 1998).

Materials and methods

Isolation of genomic DNA probes

A human genomic DNA library in PACs was screened with primers corresponding to the middle of the coding region of KCNK3 cDNA as described (Ioannou et al., 1994). These primers, 5′-GCGAGCGCATCAACACCTTGGTGAGG-3′ and 5′-GCGGCTGCGTCTGCAGGGCCTGGTC-3′, amplify a 265-bp fragment from human genomic DNA. Three PCR-positive clones verified to be KCNK3-gene specific were identified. A murine genomic DNA library in bacteriophage P1 was screened with primers corresponding to the 3′ end of the coding region of murine *Kcnk3* cDNA as described (Pierce et al., 1992) These primers, 5′-GCAGACGCAGCCGCAGTATG-3′ and 5′-GCCTGGCCGTTGTGCGTGAGCAGGG-3′, amplify a 168-bp fragment from murine genomic DNA. Three PCR-positive clones verified to be KCNK3-gene specific were identified.

In situ hybridization

Preparation of metaphase chromosomes, labeling of genomic clones with digoxigenin-11-dUTP, hybridization conditions, post hybridization washes, and probe detection were performed as previously described (Ried et al., 1992). The digoxigenin-labeled clones were co-hybridized with a biotin-labeled Alu oligonucleotide (MG-009) to generate an R-banded karyotype (Matera et al., 1992). Probes were detected with FITC-avidin and rhodamine-antidigoxigenin antibodies as described (Matera et al., 1992) Images were captured using a computer-controlled Zeiss Axioskop epifluorescence microscope coupled to a CCD camera. FITC and rhodamine signals were recorded separately as gray scale images, enhanced, pseudo-colored and merged.

Human KCNK3:
Probe names: 18531, 18532, 18533
Probe type: human genomic DNA
Insert sizes: ~ 180 kb
Vector: pADsacBII
Proof of authenticity: restriction enzyme analysis, Southern blotting, and limited DNA sequencing
Gene reference: AF006823

Supported in part by grants from the NIH and the Children's Clinical Research Center, Yale University School of Medicine.

Received 25 March 1999; manuscript accepted 15 April 1999.

Request reprints from Dr. Patrick G. Gallagher, Department of Pediatrics, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06520-8064 (USA); telephone: 203-688-2896; fax: 203-785-6974; e-mail: Patrick.Gallagher@Yale.edu

KARGER

E-mail karger@karger.ch
Fax + 41 61 306 12 34
http://www.karger.com

Accessible online at:
http://BioMedNet.com/karger

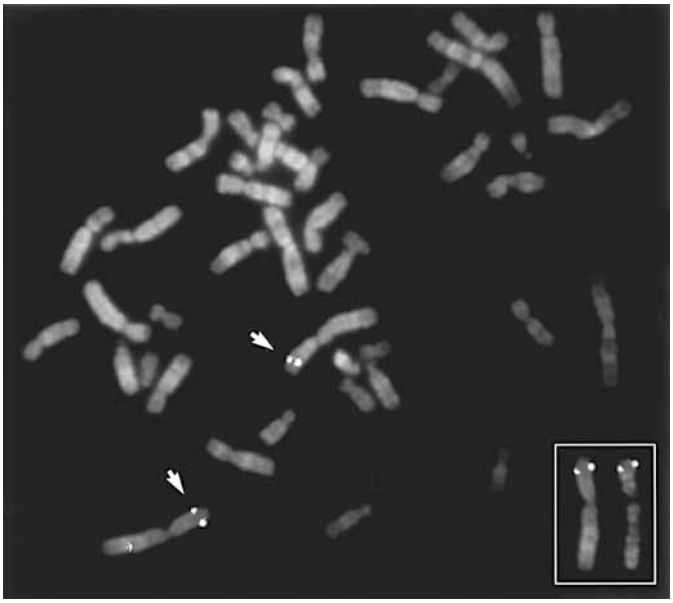

Fig. 1. Chromosome mapping of human KCNK3 by fluorescence in situ hybridization. Specific labeling was observed at 2p24.1 → p23.1 (arrows). Inset. The chromosome on the left is DAPI stained and the chromosome on the right is Alu banded.

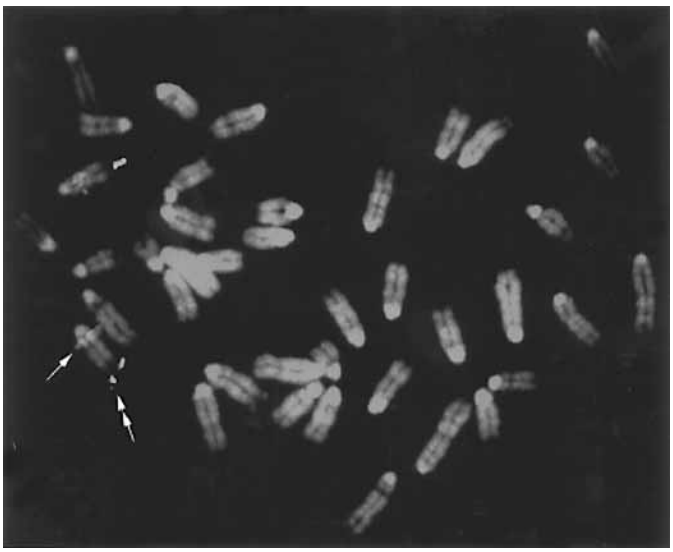

Fig. 2. Chromosome mapping of murine *Kcnk3* by fluorescence in situ hybridization. Specific labeling was observed at 5B (arrow). Localization to chromosome 5 was confirmed by co-hybridization with a murine chromosome 5 probe ATCC# 63383 (double arrow) (Mongelard et al., 1996).

Murine *Kcnk3*:
Probe names: 15456, 15457, 15458
Probe type: murine genomic DNA
Insert sizes: ~110 kb
Vector: pAd10-SacBII
Proof of authenticity: restriction enzyme analysis, Southern blotting, and limited DNA sequencing
Gene reference: AB008537

Results

Mapping results human KCNK3
Location: 2p24.1 → p23.1
Number of cells examined: 24
Number of cells with specific signal: 24
Most precise assignment: 2p24.1 → p23.1 (FLpter values 0.10–0.12)
Location of background signals (sites with >2 signals): 0

Mapping results murine Kcnk3
Location: 5B
Number of cells examined: 22
Number of cells with specific signal: 22
Most precise assignment: 5B
Location of background signals (sites with >2 signals): 0

References

Duprat F, Lesage F, Fink M, Reyes R, Heurteaux C, Lazdunski M: TASK, a human background K^+ channel to sense pH variations near physiological pH. EMBO J 16:5464–5471 (1997).

Goldstein SA, Wang KW, Ilan N, Pausch MH: Sequence and function of the two P domain potassium channels: implications of an emerging superfamily. J molec Med 76:13–20 (1998).

Ioannou PA, Amemiya CT, Garnes J, Kroisel PM, Shizuya H, Chen C, Batzer MA, deJong PJ: A new bacteriophage P1-derived vector for the propagation of large human DNA fragments. Nature Genet 6:84–89 (1994).

Jan LY, Jan YN: Cloned potassium channels from eukaryotes and prokaryotes. A Rev Neurosci 20:91–123 (1997).

Kim D, Fujita A, Horio Y, Kurachi Y: Cloning and functional expression of a novel cardiac two pore background K^+ channel. Circ Res 82:513–519 (1998).

Leonoudakis D, Gray AT, Winegar BD, Kindler CH, Harada M, Taylor DM, Chavez RA, Forsayeth JR, Yost CS: An open rectifier potassium channel with two pore domains in tandem cloned from rat cerebellum. J Neurosci 18:868–877 (1998).

Lesage F, Lazdunski M: Mapping of human potassium channel genes TREK-1 (KCNK2) and TASK (KCNK3) to chromosomes 1q41 and 2p23. Genomics 51:478–479 (1998).

Lopes CMB, Gallagher PG, Wong C, Buck M, Goldstein SAN: OATs: open, acid-sensitive, two P domain K^+ channels from mouse heart. Biophysical J 74:A44 (1998).

Matera AG, Ward DC: Oligonucleotide probes for the analysis of specific repetitive DNA sequences by fluorescence in situ hybridization. Hum Mol Genet 1:535–359 (1992).

Mongelard F, Poras I, Usson Y, Batteux B, Robert-Nicoud R, Avner P, Vourc'h C: A mouse chromosome-specific YAC probe collection for in situ hybridization. Genomics 38:432–434 (1996).

Pierce JC, Sternberg N, Sauer B: A mouse genomic library in the bacteriophage P1 cloning system: organization and characterization. Mammal Genome 3:550–558 (1992).

Ried T, Baldini A, Rand TC, Ward DC: Simultaneous visualization of seven different DNA probes by in situ hybridization using combinatorial fluorescence and digital imaging microscopy. Proc natl Acad Sci, USA 89:1388–1392 (1992).